

WHAT IS CLAIMED IS:

1. An apparatus for steering a beam of light, said apparatus comprising:

(a) an acousto-optical deflector; and

(b) a spectrally dispersive element, said spectrally dispersive element and said acousto-optical deflector being optically coupled to one another.

2. The apparatus as claimed in claim 1 wherein said spectrally dispersive element is positioned in front of said acousto-optical deflector.

3. The apparatus as claimed in claim 1 wherein said spectrally dispersive element is positioned behind said acousto-optical deflector.

4. The apparatus as claimed in claim 1 wherein said spectrally dispersive element is oriented relative to said acousto-optical deflector so that said spectrally dispersive element disperses multi-chromatic light in a direction opposite to that dispersed by said acousto-optical deflector.

5. The apparatus as claimed in claim 4 wherein said spectrally dispersive element is constructed to disperse multi-chromatic light in an amount equally opposite to, for at least a portion of said multi-chromatic light, that dispersed by said acousto-optical deflector.

6. The apparatus as claimed in claim 1 wherein said spectrally dispersive element is selected from the group consisting of a prism, a grating and a second acousto-optical deflector.

7. The apparatus as claimed in claim 6 wherein said spectrally dispersive element is a prism.

8. The apparatus as claimed in claim 5 wherein said spectrally dispersive element is positioned in front of said acousto-optical deflector, said apparatus further comprising a rotatable mirror, said rotatable mirror being optically coupled to each of said acousto-optical deflector and said spectrally dispersive element and being positioned therebetween.

9. The apparatus as claimed in claim 8 further comprising a fixed mirror, said fixed mirror being optically coupled to each of said acousto-optical deflector, said rotatable mirror and said spectrally dispersive element and being positioned between said acousto-optical deflector and said spectrally dispersive element.

10. The apparatus as claimed in claim 5 wherein said spectrally dispersive element is positioned in front of said acousto-optical deflector, said apparatus further comprising a rotatable mirror, said rotatable mirror being optically coupled to said spectrally dispersive element and being positioned in front thereof.

11. The apparatus as claimed in claim 5 wherein said spectrally dispersive element is positioned behind said acousto-optical deflector, said apparatus further comprising a rotatable mirror, said rotatable mirror being optically coupled to each of said acousto-optical deflector and said spectrally dispersive element and being positioned therebetween.

12. An apparatus for steering a beam of light, said apparatus comprising:

(a) first beam deflection means for deflecting said beam along a first axis, said first beam deflection means comprising

(i) a first acousto-optical deflector, and

(ii) a first spectrally dispersive element, said first spectrally dispersive element and said first acousto-optical deflector being optically coupled to one another, said first spectrally dispersive element being oriented relative to said first acousto-optical deflector so that said first spectrally dispersive element disperses multi-chromatic light in a direction opposite to that dispersed by said first acousto-optical deflector, said first spectrally dispersive element being constructed to

disperse multi-chromatic light, for at least a portion of said multi-chromatic light, in an amount equal to that dispersed by said first acousto-optical deflector; and

(b) second beam deflection means for deflecting said beam along a second axis, said second axis being different from said first axis, said second beam deflection means comprising

(i) a second acousto-optical deflector, and

(ii) a second spectrally dispersive element, said second spectrally dispersive element and said second acousto-optical deflector being optically coupled to one another, said second spectrally dispersive element being oriented relative to said second acousto-optical deflector so that said second spectrally dispersive element disperses multi-chromatic light in a direction opposite to that dispersed by said second acousto-optical deflector, said second spectrally dispersive element being constructed to disperse multi-chromatic light, for at least a portion of said multi-chromatic light, in an amount equal to that dispersed by said second acousto-optical deflector.

13. The apparatus as claimed in claim 12 wherein said second axis is perpendicular to said first axis.

14. The apparatus as claimed in claim 12 wherein said first beam deflection means is constructed to scan said beam over a plurality of contiguous locations along said first axis and wherein said second beam deflection means is constructed to scan said beam over a plurality of contiguous locations along said second axis.

15. The apparatus as claimed in claim 12 wherein said first beam deflection means is constructed to scan said beam over a plurality of non-contiguous locations long said first axis and wherein said second beam deflection means is constructed to randomly deflect said beam over a plurality of contiguous locations along said second axis.

25. The method as claimed in claim 24 wherein said light has a wavelength in the range of about 400 to 700 nm.

26. The method as claimed in claim 24 wherein said light has a wavelength in the range of about 700 to 1000 nm.

27. The method as claimed 16 wherein said step of passing said beam of light through a spectrally dispersive element is performed before said step of deflecting said beam of light using an acousto-optical deflector.

28. The method as claimed 16 wherein said step of passing said beam of light through a spectrally dispersive element is performed after said step of deflecting said beam of light using an acousto-optical deflector.

29. The method as claimed in claim 16 wherein said spectrally dispersive element is a prism.

30. An apparatus for spectrally dispersing multi-chromatic light traveling along a first axis, said apparatus comprising:

(a) a spectrally dispersive element, disposed along said first axis, for dispersing said multi-chromatic light;

(b) a pair of mirrors optically coupled to said spectrally dispersive element and positioned thereafter to redirect said dispersed light along said first axis.

31. The apparatus as claimed in claim 30 wherein one of said pair of mirrors is a rotatable mirror to adjust for wavelength-dependent variations in the deflection of said dispersed light.

32. The apparatus as claimed in claim 31 wherein the other of said pair of mirrors is a fixed mirror.

33. The apparatus as claimed in claim 31 further comprising means for rotating said rotatable mirror.

34. The apparatus as claimed in claim 33 wherein said rotating means comprises a rotatably mounted arm and a motor for rotating said rotatably mounted arm, said rotatable mirror being fixedly mounted on said rotatably mounted arm.

35. The apparatus as claimed in claim 34 wherein said motor is controllable by computer.

36. The apparatus as claimed in claim 34 further comprising a base, said spectrally dispersive element, said pair of mirrors, said rotatably mounted arm and said motor being mounted on said base.

37. A method of imaging a sample using multi-photon excited fluorescence laser scanning microscopy, said method comprising the steps of:

(a) providing a sample containing fluorescent molecules which radiate photons of a first characteristic energy;

(b) producing a scanning beam of ultrashort laser light pulses, said scanning beam producing step comprising

(i) providing a beam of ultrashort laser light pulses comprising photons of a second characteristic energy, wherein said second characteristic energy is less than said first characteristic energy and wherein the simultaneous absorption of a plurality of said photons of said second characteristic energy by said fluorescent molecules causes the fluorescence of said fluorescent molecules,

(ii) passing said beam through a spectrally dispersive element, and

(iii) deflecting said beam using an acousto-optical deflector,

(iv) wherein said spectrally dispersive element is oriented to disperse multi-chromatic light in a direction opposite to that dispersed by said acousto-optical deflector;

(c) focusing said scanning beam at a focal point within said sample to produce an illumination intensity sufficiently high only at said focal point to produce molecular excitation and fluorescence of said sample by simultaneous absorption of a plurality of incident photons;

(d) detecting the fluorescence produced by said sample; and

(e) using the detected fluorescence to form an image of the sample.

38. The method as claimed in claim 37 wherein said spectrally dispersive element is constructed to disperse multi-chromatic light, for at least a portion of said multi-chromatic light, in an amount equal to that dispersed by said acousto-optical deflector.

39. The method as claimed in claim 37 wherein said step of passing said beam through a spectrally dispersive element is performed prior to the step of deflecting said beam using an acousto-optical deflector.

40. A multi-photon excited fluorescence laser scanning microscope for forming a magnified image of a sample, said sample containing fluorescent molecules which radiate photons of a first characteristic energy, said multi-photon excited fluorescence laser scanning microscope comprising:

(a) means for producing a scanning beam of ultrashort laser light pulses, said scanning beam producing means comprising

(i) a laser source for providing a beam of ultrashort laser light pulses comprising photons of a second characteristic energy, wherein said second characteristic energy is less than said first characteristic energy and wherein the simultaneous absorption of a plurality of

said photons of said second characteristic energy by said fluorescent molecules causes the fluorescence of said fluorescent molecules,

(ii) a first acousto-optical deflector optically coupled to said laser source for scanning said beam along a first axis,

(iii) a first spectrally dispersive element optically coupled to said first acousto-optical deflector, said first spectrally dispersive element being oriented relative to said first acousto-optical deflector so as to disperse multi-chromatic light in a direction opposite to that dispersed by said first acousto-optical deflector;

(b) means for focusing said scanning beam to a focal point within said sample to produce an illumination intensity sufficiently high only at said focal point to produce molecular excitation and fluorescence of said sample by simultaneous absorption of at least two incident photons;

(c) means for detecting the fluorescence produced by said sample; and

(d) means for using the detected fluorescence to form a magnified image of the sample.

41. The multi-photon excited fluorescence laser scanning microscope as claimed in claim 40 wherein said first spectrally dispersive element is constructed to disperse multi-chromatic light, for at least a portion of said multi-chromatic light, in an amount equal to that dispersed by said first acousto-optical deflector.

42. The multi-photon excited fluorescence laser scanning microscope as claimed in claim 40 wherein said scanning beam producing means further comprises means for scanning the sample in a direction perpendicular to said first axis.

43. The multi-photon laser scanning microscope as claimed in claim 42 wherein said means for scanning the sample in a direction perpendicular to said first axis comprises a second acousto-optical deflector and a second spectrally dispersive element, said second spectrally dispersive element being oriented relative to said second acousto-optical deflector so as to disperse multi-chromatic light in a direction opposite to that dispersed by said second acousto-optical deflector.

44. A laser scanning microscope for forming a magnified image of a sample, the sample containing fluorophores, said laser scanning microscope comprising:

(a) means for producing a scanning beam of light pulses, said scanning beam producing means comprising:

(i) means for providing a beam of light pulses, said light pulses being of a wavelength suitable to excite said fluorophores,

(ii) a first acousto-optical deflector optically coupled to said beam providing means for scanning said beam along a first axis,

(iii) a first spectrally dispersive element optically coupled to said first acousto-optical deflector, said first spectrally dispersive element being oriented relative to said first acousto-optical deflector so as to disperse multi-chromatic light in a direction opposite to that dispersed by said first acousto-optical deflector;

(b) means for focusing said scanning beam to a focal point within said sample;

(c) means for detecting the fluorescence produced by said sample; and

(d) means for using the detected fluorescence to form a magnified image of the sample.

45. The laser scanning microscope as claimed in claim 44 wherein said first spectrally dispersive element is positioned between said beam providing means and said first acousto-optical deflector.

46. A method of imaging a sample using multi-harmonic generation laser scanning microscopy, said method comprising the steps of:

(a) providing a sample, the sample containing molecules having the appropriate nonlinear susceptibility;

(b) producing a scanning beam of ultrashort laser light pulses, said scanning beam producing step comprising

(i) providing a beam of ultrashort laser light pulses comprising photons of a first wavelength capable of interacting with said molecules having the appropriate nonlinear susceptibility to create, by multi-harmonic generation, photons of a second wavelength,

(ii) passing said beam through a spectrally dispersive element, and

(iii) deflecting said beam using an acousto-optical deflector,

(iv) wherein said spectrally dispersive element is oriented to disperse multi-chromatic light in a direction opposite to that dispersed by said acousto-optical deflector;

(c) focusing said scanning beam at a focal point within said sample to produce an illumination intensity sufficiently high only at said focal point to generate, by multi-harmonic generation, photons of said second wavelength;

(d) detecting the photons of said second wavelength emitted from said sample; and

(e) using the detected photons of said second wavelength to form an image of the sample.

47. The method as claimed in claim 46 wherein said step of passing said beam through a spectrally dispersive element is performed prior to said step of deflecting said beam using an acousto-optical deflector.

48. A multi-harmonic generation laser scanning microscope for forming a magnified image of a sample, the sample containing molecules having the appropriate nonlinear susceptibility, said multi-harmonic generation laser scanning microscope comprising:

(a) means for producing a scanning beam of ultrashort laser light pulses, said scanning beam producing means comprising

(i) a laser source for providing a beam of ultrashort light pulses comprising photons of a first wavelength capable of interacting with said molecules having the appropriate nonlinear susceptibility to create, by multi-harmonic generation, photons of a second wavelength,

(ii) a first acousto-optical deflector optically coupled to said laser source for scanning said beam along a first axis,

(iii) a first spectrally dispersive element optically coupled to said first acousto-optical deflector, said first spectrally dispersive element being oriented relative to said first acousto-optical deflector so as to disperse multi-chromatic light in a direction opposite to that dispersed by said first acousto-optical deflector;

(b) means for focusing said scanning beam at a focal point within said sample to produce an illumination intensity sufficiently high only at said focal point to generate, by multi-harmonic generation, photons of said second wavelength;

(c) means for detecting the photons of said second wavelength emitted from said sample; and

(d) means for using the detected photons of said second wavelength to form an image of the sample.

49. A laser scanning microscope, said laser scanning microscope comprising:

(a) means for producing a scanning beam of laser light, said scanning beam producing means comprising

(i) a laser source for providing a beam of laser light,

(ii) a first acousto-optical deflector optically coupled to said laser source for scanning said beam along a first axis,

(iii) a first spectrally dispersive element optically coupled to said first acousto-optical deflector, said first spectrally dispersive element being oriented relative to said first acousto-optical deflector so as to disperse multi-chromatic light in a direction opposite to that dispersed by said first acousto-optical deflector;

(b) means for focusing said scanning beam at a focal point within a sample;

(c) confocal fluorescence detection means for detecting single-photon fluorescence emitted from said sample;

(d) non-confocal fluorescence detection means for detecting multi-photon fluorescence emitted from said sample;

(e) multi-harmonic generation detection means for detecting multi-harmonic generation photons emitted from said sample; and

(f) means, coupled to said confocal fluorescence detection means, said non-confocal detection means and said multi-harmonic generation detection means, for forming an image of said

sample using at least one of the detected single-photon fluorescence, the detected multi-photon fluorescence and the detected multi-harmonic generation photons.

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